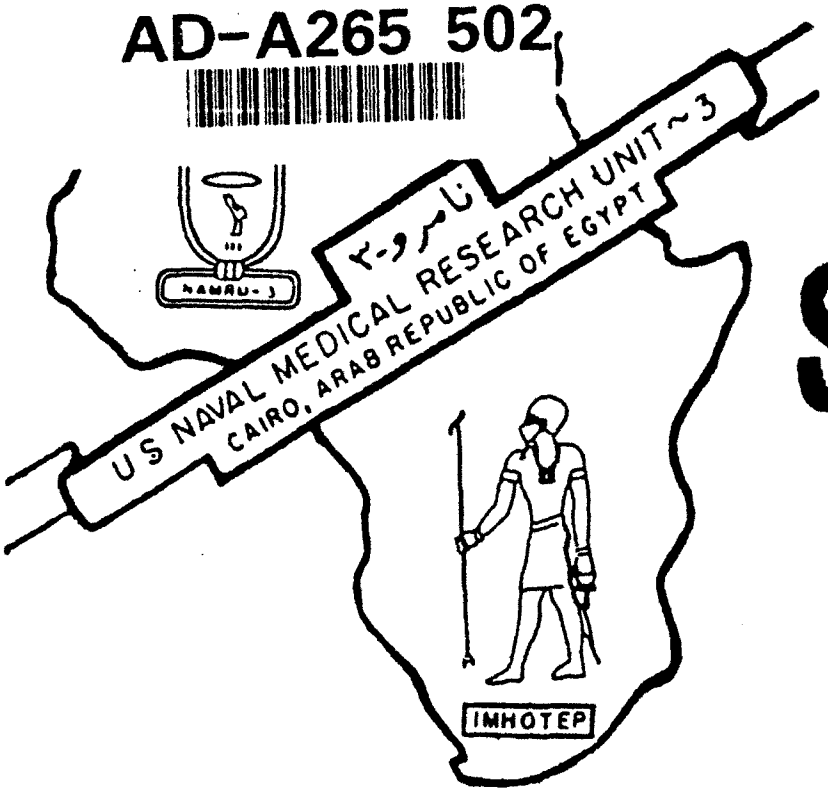

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MONITORING FOR HIV-1, HIV-2, HTLV-I SERO-PROGRESSION AND SERO-CONVERSION IN A POPULATION AT RISK IN EAST AFRICA

BY

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ABSTRACT

Thirty-three individuals from East Africa, at risk for acquiring sexually transmitted infections, were selected to be monitored over a five month period for evidence of sero-progression and/or sero-conversion for human immunodeficiency virus type-1 and type-2 (HIV-1, HIV-2), and human T cell leukemia virus type-1 (HTLV-I). Initially, all sera were reactive by at least one retroviral screening assay, but most produced negative or indeterminate results by western blot assays. Five months after the initial screening, western blot assays indicated that one individual exhibited full sero-conversion for HIV-1; one HIV-1 positive individual also became positive for HIV-2; and two subjects showed sero-progression to become HTLV-I confirmed positive. Sera from fourteen individuals produced indeterminate results by western blot for HIV-1, ten of which were previously negative; the remaining four sera exhibited reactivity to at least one additional viral specific antigen after the five months. Circulating HIV-1 antigen was not demonstrated in any of the sera but DNA isolated from one of the individuals with indeterminate results produced a positive reaction for HIV-1 by the polymerase chain reaction.

INTRODUCTION

Infection by human immunodeficiency virus (HIV) leads to the production of virus specific antibodies usually detectable by 12 weeks following exposure (Gaines et al., 1987). However, development of the antibody response depends on many factors and may require longer time periods (Ranki et al., 1987). Presently, available HIV antibody assays often yield results which are considered inconclusive or indeterminate by current criteria. Indeterminate results by the confirmatory western blot assay are difficult to interpret, and determination of HIV infection may require many months before confirmation can be verified (update MMWR,

1988). It has been suggested that subjects with such indeterminate results should be retested after 3 - 6 months for evidence of sero-conversion. Subsequent non-progressing western blot profiles are then suggestive that the individual has not been infected with HIV. Further interpretative problems arise when an individual's serum is reactive by a screening assay, but negative by confirmatory assays. Although these subjects are considered negative for HIV infection, the screening assays may be more sensitive than confirmatory assays (Burke and Redfield, 1986) and therefore the HIV immune status of these individuals is questionable, and their blood is considered unsafe for transfusion (CDC MMWR, 1985). Assays to detect antigen in serum (Deinhardt, 1988), and DNA from lymphocytes by amplification (Loche and Mach, 1988) have been implemented in an effort to detect early infection.

We have examined sera from high risk individuals in Djibouti for evidence of exposure to retroviruses over a 12 month period (Constantine et al., 1989, Fox et al., 1988, Fox et al., 1989a, and Fox et al., 1989b). In June 1988 (Constantine et al., 1989), sera from 599 individuals were tested using five screening assays for HIV-1, and by assays for HIV-2 and human T-lymphotropic virus type I (HTLV-I). At that time, sera from 64 subjects reacted by at least one of the assays and therefore were further characterized by western blot analysis for the three viruses. All but seven of these sera exhibited indeterminate or negative reactions by blot assays, and therefore the actual antibody status of many subjects was uncertain.

In November 1988, we monitored a portion of this high risk population for evidence of quantitative or qualitative change in antibody reactivity to viral antigens (sero-progression) or sero-conversion for HIV-1, HIV-2, and HTLV-I. In addition, indeterminate sera were tested for circulating antigen, and lymphocytes for evidence of retroviral DNA in an effort to reveal early infection in subjects. Since all of these retroviruses are transmitted sexually, it was also of interest to determine if any of the high risk individuals might have acquired concurrent retroviral infections.

MATERIAL AND METHODS

A total of 33 individuals were available for follow-up testing in November 1988. All were from young adult female prostitutes in the East African city of Djibouti. Details on the study population were described elsewhere (Fox et al., 1989). All individuals had been tested in June 1988 for HIV-1, HIV-2, and HTLV-I antibodies as part of a national survey (Constantine et al., 1989). At that time, sera from all individuals were reactive by at least one of seven retroviral screening assays but only seven sera met the criteria required to be confirmed positive by western blot analysis (5 HIV-1, and 2 HTLV-I).

Sera for follow-up were tested by three western blot assays : HIV-1, HIV-2, and HTLV-I (Du Pont, EI DuPont de Nemours, Wil, De). HIV-1 and HIV-2 Kits were of the same lot as those used in June. Our minimum criteria for positivity included reactivity to at least p24, gp41, and gp120/160 for HIV-1; gp41 and gp 120/160 for HIV-2; and p19, p24 and gp46 for HTLV-I. Sera which yielded reactivity intensities less than that observed for the corresponding weak positive controls (cut off values) were considered as indeterminate, even if the criteria were met qualitatively. Also, sera that produced reactivity to one or more virus specific bands, but failed to meet the minimum criteria, were labeled as indeterminate. Sera were considered negative if no viral specific bands were detected.

A capture ELISA (Abbott Laboratories, North Chicago, IL.) to detect circulating antigen in sera was performed on all samples. Samples reacting positive were further subjected to neutralization for confirmation of antigenemia. Sera yielding indeterminate results by western blot were assayed by an indirect immunofluorescent assay (IFA) designed to detect antibody to HIV-1 (Electronucleonics, Inc., Columbia Maryland). A 1+ or greater reaction at a screening dilution of 1 : 20 was considered positive. In addition, lymphocyte DNA isolated from these patients with indeterminate results was analyzed by the polymerase chain reaction (PCR) technique using a primer pair (SK 38/39)

specific for the p24 nucleotide sequence of HIV-1. Details of the PCR procedure are published elsewhere (Ou et al., 1988).

RESULTS

Negative Western Blot Sera

Of the 33 sera collected in November 1988, nine produced no observable reactions by any of the three western blot assays. These same sera had also been negative by western blot assays in June.

Positive HIV-1 Western Blot Sera

Sera from six individuals met the criteria for HIV-1 confirmed positive. Four of these exhibited no change in their HIV-1 reactions from June, while one showed an increase in reaction intensity. The remaining serum exhibited full sero-conversion from its negative western blot result in June; it had been initially identified by the Fujirebio HIV-1 screening assay. None of the six sera produced any significant reactions when tested by the HIV-2 or HTLV-I blot assays.

Indeterminate Labeled Sera

Sera from fourteen individuals produced indeterminate results by the HIV-1 western blot; ten of these had been completely negative in June. Four indeterminate sera, which also had been indeterminate in June, showed reactivity to at least on additional HIV-1 viral specific antigen in November. The western blot profiles of indeterminate sera observed in June and November and the initially reactive screening assays are presented in Table 1. Some of these sera produced minor bands by the HIV-2 and HTLV-I blots, but reactions and patterns were

unremarkable. In November, lymphocyte DNA from one of these individuals with indeterminate results (G-131) was positive for HIV-1 by PCR; the serum was initially detected as reactive by the Elavia screening ELISA in June. Lymphocyte DNA from another individual produced equivocal results by PCR in November.

Positive HIV-2 Western Blot Sera

One serum from a previously confirmed HIV-1 positive individual was also reactive in a screening assay for HIV-2 in June, but was indeterminate by the HIV-2 western blot. However, this serum was clearly positive in November, as evident by a strong reaction to the envelope antigens of HIV-2.

Positive HTLV-I Western Blot Sera

Sera from two individuals were reactive for HTLV-I by a screening assay, but not by a confirmatory assay in June; both were positive by the HTLV-I western blot in November. Two additional individuals remained confirmed positive for HTLV-I from June, while none of the four sera produced significant reactions by the HIV-1 or HIV-2 blot assays.

HIV-1 Antigen Assay

Several sera produced positive reactions by the antigen capture assay but could not be confirmed by neutralization.

IFA

Despite non-specific reactions by a few sera, only one of the fourteen indeterminate-labeled sera (G-56) produced a 1-2+ reaction at a titer of 20 by

IFA. This serum was initially reactive one-time only by the Abbott recombinant ELISA and was from a patient who subsequently acquired pulmonary tuberculosis.

PCR

Of the indeterminate sera, only one was found to be positive by PCR. Another serum produced equivocal results (Table 1).

DISCUSSION

Approximately half of the prostitutes (n = 33), whose sera produced some reactivity by retroviral assays in June, were available after 5 months to be monitored for evidence of sero-progression and/or sero-conversion for HIV-1, HIV-2, and HTLV-I. One previously HIV-1 weak positive serum displayed an increased intensity of reaction, and one subject sero-converted completely by western blot for HIV-1. Two subjects were considered to show sero-progression from HTLV-1 reactive by screening, to become HTLV-I confirmed positive. One HIV-1 positive individual acquired antibodies to the envelope antigens of HIV-2, suggesting a concomitant or dual infection, although cross reacting antibodies to envelope antigens of HIV-1 and HIV-2 have recently been reported (Tedder et al., 1988).

Fourteen individuals with negative or indeterminate results by the HIV-1 western blot in June were considered to show some sero-progression for HIV-1 by November. Six of these had serological profiles highly suggestive of exposure to HIV-1. Since results from these sera still did not meet our minimum criteria for positivity, and since the interpretation of indeterminate profiles has not been established, the status of these individuals regarding HIV-1 infection remains uncertain. However, if our western blot criteria for positivity had been less stringent (i.e. positivity defined as reactivity to any two of the three major group

antigens) more than half (Fox et al., 1989b) of these sera would now have been labeled as HIV-1 confirmed seropositive, indicating a sero-conversion rate of 32% (9/28) for HIV-1.

HIV antigen was not confirmed in any of the 33 sera, and therefore the diagnosis of early HIV infection by demonstrating antigenemia could not be accomplished. However, HIV antigenemia in Africans with HIV infection has been reported to be only 1% (Girard, 1988), and the sensitivity of the assay for detecting antigen in serum is low (Allain et al., 1986). The PCR identified one, and perhaps two, of the individuals with indeterminate serologic results as having viral DNA, indicating HIV-1 infection. The western blot profiles of these two individuals were also suggestive of HIV-1 infection.

The question arises whether the screening assays which initially detected the reactive (non-confirmed) sera were in fact more sensitive than the western blot, or if the results reflected false positive reactions at that time. The data probably favor the former, since in many cases sero-progression was revealed by the western blots, and in at least one case HIV-1 infection was detected by PCR. Of the six sera showing the most dramatic HIV-1 serological progression, HIV-1 western blot profiles were compatible with early infection, although not definitive. In addition, one of these sera (G56) also was positive by the IFA and the subject had developed tuberculosis, a disease common among HIV infected individuals in Africa.

In only one case did a screening assay for HIV-1 (Fujirebio gelatin agglutination) correctly identify an individual who subsequently sero-converted by western blot in November. This particular assay has been reported by us (Constantine et al., 1989) and others (Sng et al., 1988) to be more sensitive than some other HIV-1 screening tests. Similarly, the individuals who sero-converted for HTLV-I had been initially identified in June only by the HTLV-I screening assay (Fujirebio gelatin agglutination), and the HIV-2 sero-converter had been initially detected only by an HIV-2 screening assay (Genetic Systems). As noted, in all cases of sero-conversion, the antibodies in the sera were previously detected

by their homologous screening assay; indicating that any one retroviral screening assay may not be adequate to identify other retroviral infections.

In summary, our study of a high risk population, previously having inconclusive retroviral serological results, identified HIV-1, HTLV-I, and perhaps HIV-2 sero-converters. A total of eighteen individuals (55%), including one with PCR positive results, sero-progressed or sero-converted for one of the retroviruses over the 5 month period. Only nine of twenty-six individuals (35%) remained completely negative for antibodies to all three retroviruses by western blot assays.

Sero-conversion confirms viral infection, while sero-progression may represent procession toward sero-conversion. There is a need to monitor individuals whose sera cannot be characterized as positive or negative for retroviral infection, and also a need to define the correct meaning of inconclusive serologic results. In addition, individuals whose sera initially react by any retroviral screening assay should be followed for evidence of future sero-progression and sero-conversion for HIV and HTLV-I infections.

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Table 1. HIV-1 sero-progression, as identified by western blot, and PCR results for fourteen individuals with inconclusive serological results over a four month period.

HIV-1 Western Blot Profile				
Serum #	Initial positive screening test	June	November	PCR
G-51	GS	-	15,24	-
G-55	FI	-	15,41,51	-
G-76	A	-	24,55	-
G-87	FI	-	24,55,66	-
G-99	F	-	120	-
G-106	FI	-	15,24,66	-
G-109	GS	-	17,24,66,120*	-
G-156	A	-	15,17,24,55,66	-
W-56	E	-	24,55	-
W-81	FI	-	24,55	-
G-56	A	17,24	17,24,31,51,55,66,160*	-
G-131	E	55	24,55	+
W-22	A,E,F	24,55	24,41,55*	-
W-134	E,C	24,55	24,31,41,51,55,66*	+/-

+ = positive

+/- = indeterminate

- = negative

* = Weak intensity bands

A = Abbott recombinant ELISA

C = Cambridge latex agglutination

E = Elavia viral lysate ELISA

F = Fujirebio HIV-1 gelatin agglutination

FI = Fujirebio HTLV-I gelatin agglutination

GS = Genetic Systems HIV-2 viral lysate ELISA

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